ABSTRACT

Chimeric Antigen Receptor (CAR) T cell therapy has revolutionised immunotherapy by boosting T cell-driven tumour cell destruction through specific antigen recognition. Despite its success in haematological cancers, significant hurdles in using CAR T cells for solid tumours are the suppression of CAR T cell proliferation and effector functions. This research addresses the pressing challenge in cancer treatment by proposing a strategy to enhance CAR T cell therapy's efficacy in solid tumours. Focusing on CD45 and PD-1, known suppressors in the tumour microenvironment, CRISPR/Cas9 technology was employed to precisely knockout these genes in T cells. The study optimised sgRNA design, nucleotide delivery, and production protocols, successfully generating CD45 and PD-1 negative "enhanced" T cells. Notably, the study also explored the impact of PD-1 knockout on exhaustion markers, revealing stable expressions of LAG3 and TIGIT but dynamic regulation of TIM3. These findings contribute to refining a scalable protocol for integrating immune checkpoint modulation into CAR T cell therapy, potentially improving therapeutic outcomes in solid tumour treatment.

Keywords: CD45, PD-1, CAR T cell, CRISPR/Cas9, gene knockout, optimisation